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EXAMINER

STEADMAN, DAVID J

ART UNIT

PAPER NUMBER

1652

DATE MAILED: 09/17/2003

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Please find below and/or attached an Office communication concerning this application or proceeding.

**Office Action Summary**

Application No.

09/743,731

Applicant(s)

SMIT, JOHN

Examiner

David J Steadman

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

- 1) ☒ Responsive to communication(s) filed on 14 July 2003.
- 2a) ☒ This action is **FINAL**.                      2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

- 4) ☒ Claim(s) 1-6 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1-6 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

**Application Papers**

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- 11) ☐ The proposed drawing correction filed on \_\_\_\_\_ is: a) ☐ approved b) ☐ disapproved by the Examiner.
- If approved, corrected drawings are required in reply to this Office action.
- 12) ☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. §§ 119 and 120**

- 13) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All b) ☐ Some \* c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
  2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  3. ☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- \* See the attached detailed Office action for a list of the certified copies not received.
- 14) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).
- a) ☐ The translation of the foreign language provisional application has been received.
- 15) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

**Attachment(s)**

- |  |   |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)                  | 4) <input type="checkbox"/> Interview Summary (PTO-413) Paper No(s). _____  |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)         | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ | 6) <input type="checkbox"/> Other: _____                                    |

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**DETAILED ACTION**

***Status of the Application***

- [1] Claims 1-6 are pending in the application.
- [2] Applicant's cancellation of claims 7-8 and amendment to claims 1-2 in Paper No. 19, filed July 14, 2003, is acknowledged.
- [3] It is noted that claims 5 and 6, previously withdrawn from consideration as being improper multiple dependent claims, are being examined on the merits. Applicant notes that a preliminary amendment corrected claim dependency. However, this amendment was not entered prior to examination on the merits. The preliminary amendment will be entered.
- [4] Applicant's arguments filed in Paper No. 19 have been fully considered and are deemed to be persuasive to overcome some of the rejections and/or objections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.
- [5] The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

***Claim Objections***

- [6] Claim 1 is objected to in the recitation of "at lease one". It is suggested that the term be replaced with "at least one". Appropriate correction is required.

***Claim Rejections - 35 USC § 112, First Paragraph***

- [7] Claims 1-6 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection. Claim 1 (claims 2-6 dependent therefrom) has been amended to recite "a *Caulobacter crescentus* S-layer protein fragment incapable of adhesion to a *Caulobacter crescentus* cell surface" (lines

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3-4). MPEP § 2163 states, "when filing an amendment an applicant should show support in the original disclosure for new or amended claims" and "[i]f the originally filed disclosure does not provide support for each claim limitation, or if an element which applicant describes as essential or critical is not claimed, a new or amended claim must be rejected under 35 U.S.C. 112, para. 1, as lacking adequate written description". The examiner has carefully reviewed applicant's asserted support for amended claim 1. However, the examiner can find no support for "a *Caulobacter crescentus* S-layer protein fragment incapable of adhesion to a *Caulobacter crescentus* cell surface" in applicant's asserted support for amended claim 1.

**[8]** The scope of enablement rejection of claims 1-6 under 35 U.S.C. 112, first paragraph, is maintained for the reasons of record and the reasons stated below. The rejection was fully explained in a previous Office action (see item 4 of Paper No. 17). Applicant argues (beginning at the bottom of page 4 of Paper No. 19) claim 1 has been amended to recite not merely any part of a *Caulobacter* C-terminal secretion signal but a *Caulobacter crescentus* S-layer protein fragment incapable of adhesion to a *Caulobacter crescentus* cell surface and including a secretion signal. Applicant cites support in the specification for such fragments and argues the art provides adequate guidance as to how to select such an S-layer protein fragment that can be used to practice the claimed invention. Applicant argues the examiner's conclusion that a fusion protein must contain at least amino acids 905-1026 in order to precipitate is beyond what is supported by the reference of Smit et al. as, applicant alleges, some of the fusion proteins containing amino acids 945-1026, 943-1026, and 904-1026 may also be able to be secreted and precipitate, which can be readily determined according to the method of Smit et al. Applicant argues that it is not necessary to test all species covered by claim 1 to demonstrate operativeness and cites *In re Angstadt and Griffin*, 190 USPQ 214 (CCPA 1976) in support of their argument. Applicant argues that the guidance provided in the specification and the prior art would enable a skilled artisan to determine those fragments of a *Caulobacter crescentus* S-layer protein that can be used to practice the claimed method without undue experimentation. Applicant's argument is not found persuasive.

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It is the examiner's position that undue experimentation would be required for a skilled artisan to make the entire scope of the claimed invention. The examiner acknowledges that claim 1 has been amended to recite a *Caulobacter crescentus* S-layer protein fragment incapable of adhesion to a *Caulobacter crescentus* cell surface and including a secretion signal. However, there remains a high degree of unpredictability in determining which of those fragments of a *Caulobacter crescentus* S-layer protein would be useful for practicing the claimed method. It is noted that applicant has disclosed only the representative examples of amino acids 622, 690, 784, 892, and 907 to 1026 of *Caulobacter crescentus* S-layer protein as fragments that can be successfully employed in the claimed method. In the instant case, applicant demonstrates by way of working example the use of *only* amino acids 690-1026 (Examples 1-3, pages 15-17 of the specification) and amino acids 784-1026 (Example 4, pages 17-18 of the specification) of *Caulobacter crescentus* S-layer protein that can be used to practice the claimed method. It is noted that in *In re Angstadt and Griffin*, 190 USPQ 214 (CCPA 1976), appellants demonstrated forty different reactions by working example – in contrast to applicant's two working examples in the instant application. It is noted that, while the disclosed S-layer protein fragments are demonstrated in the specification as being useful for practicing the claimed method, the claims are not so limited to those disclosed fragments. In fact, the claims broadly encompass *any* *Caulobacter crescentus* S-layer protein fragment incapable of adhesion to a *Caulobacter crescentus* cell surface and including a secretion signal. One of skill in the art would recognize that a "fragment" of a protein can be any number of amino acids of that protein, including a single amino acid. Thus, the claims encompass a vast array of possible *Caulobacter crescentus* S-layer protein fragments. Furthermore, as stated above, there is a high degree of unpredictability that *any* fragment will have the characteristics of being insoluble and incapable of adhesion to a *Caulobacter crescentus* cell surface. Smit et al. teach that a fusion protein comprising amino acids 944-1026 of a *Caulobacter crescentus* S-layer protein was unable to precipitate (column 17, lines 19-21). Thus, the prior art provides evidence supporting the unpredictability that *any* fragment of a *Caulobacter crescentus* S-layer protein would have the desired characteristics. Applicant's statements further indicate that determining which fragments are able to precipitate is highly unpredictable as the

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data of Smit et al. would suggest that amino acids 905-1026 are required for precipitation, however, applicant states that some of the fusion proteins containing amino acids 943-1026 and 945-1026 may also be able to be secreted and precipitate (page 5 of Paper No. 19). However, there is no evidence or guidance presented in the specification or the prior art to suggest that such fragments would have the ability to precipitate – thus providing support for the examiner's argument that it is highly unpredictable as to which fragments are insoluble. Thus, it appears that certain fragments of a *Caulobacter crescentus* S-layer protein are unable to precipitate and neither the specification nor the prior art provides guidance as to which amino acids of a *Caulobacter crescentus* S-layer protein are necessary for insolubility. As such, an undue amount of experimentation would be required to determine those fragments of a *Caulobacter crescentus* S-layer protein that can be used to practice the claimed invention. Therefore, due to the broad scope of the claimed methods, the lack of guidance and working examples, the high degree of unpredictability as supported by the prior art, and the amount of experimentation required, the specification does not enable the entire scope of the claimed invention.

***Claim Rejections - 35 USC § 103***

[9] Claims 1-6 are under 35 U.S.C. 103(a) as being unpatentable over Smit et al. (US Patent 5,976,864; IDS reference AA of Paper No. 13) in view of Nomellini et al. (*J Bacteriol* 179 :6349-6354), Ausubel et al. (*Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., 1994; IDS reference AR of Paper No. 16), and Better (US Patent 5,851, 802). Claim 1 is drawn to a method of cleaving a fusion protein, which is insoluble in a medium, into a first component comprising: a) a *Caulobacter crescentus* S-layer protein fragment incapable of adhesion to a *Caulobacter crescentus* cell surface and b) a secretion signal and a second component heterologous to *Caulobacter*, wherein the fusion protein comprises at least one aspartate-proline dipeptide at a site of cleavage, wherein the method comprises combining the fusion protein with an acid solution of a strength insufficient to solubilize the fusion protein for a time sufficient for cleavage of the fusion protein at said site of cleavage and wherein the first component remains insoluble in said acid solution after cleavage. Claim 2 limits the second component of

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the method of claim 1 to becoming soluble in the acid solution after cleavage. Claims 3 and 4 limit the pH of the acid solution of claim 1 to 1.5-2.5 or 1.65-2.35, respectively. Claim 5 limits the temperature of the method of claim 1 to about 30 to 50 degrees Celsius. Claim 6 is drawn to the method of claim 1 further comprising separating the products cleaved from the fusion protein.

Smit et al. teach DNA constructs for expression and secretion of a *Caulobacter crescentus* S-layer protein (referred to as "RsaA" by Smit et al.) fused to a heterologous protein (abstract). Smit et al. teach numerous advantages for generating a fusion protein using a *Caulobacter crescentus* S-layer protein over existing fusion protein expression systems including the ubiquitous and non-pathogenic nature of *Caulobacter* and relatively high expression levels (column 2). Smit et al. teach methods for creating expression vectors encoding a *Caulobacter crescentus* S-layer protein and using said vectors for expression and secretion of fusion proteins (Examples 1-8). Smit et al. teach "[i]t may also be desirable to use *Caulobacter* strains which... shed the S-layer protein upon secretion and do not form an intact S-layer" and "[e]xamples of shedding strains are CB15Ca5 and CB15Ca10... and the smooth lipopolysaccharide deficient mutants" (column 4, lines 34-52). Smit et al. provide a specific example of using such S-layer shedding mutants for expression of a fusion protein comprising amino acids 782-1026 of a *Caulobacter crescentus* S-layer protein (column 16-17), which is disclosed as being a fusion protein that Smit et al. teach as having the ability to precipitate in culture medium (column 17, top). The fusion protein of Smit et al. does not have an aspartate-proline dipeptide for cleaving the fusion protein under acidic conditions.

Nomellini et al. teach that RsaA can be expressed as a precipitated protein that is resistant to acid solubilization. For example, Nomellini et al. teach "[w]hen it was desirable to test crystallization capability for proteins that were not crystallized and attached to the *C. crescentus* cell surface, that is, they were produced from a "shedding" strain of *Caulobacter*, an alternate method [of purification other than acid solubilization] was used. In such cases RsaA was a precipitated protein; in the case of the shedding strains, the protein apparently makes an aberrant attempt to crystallize" (page 6350, left column under "S-layer protein (RsaA)". Nomellini et al. also teach, "RsaA protein that has been shed from

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cells forms a loose precipitate, composed of fibrils of RsaA protein, in liquid culture medium. This process was apparently an aberrant attempt to crystallize and occurs in particular in S-layer shedding mutants, which lack the [smooth lipopolysaccharide] required for S-layer attachment. This precipitate was readily collected in nearly pure form but was refractory to the low-pH solubilization" (page 6352, left column, middle).

At the time of the invention, acid hydrolysis of a fusion protein at an aspartyl-prolyl (Asp-Pro) bond was well known in the prior art. For example, Ausubel et al. teach guidelines for cleavage of fusion proteins by hydrolysis at low pH (pages 16.4.13-16.4.14). Ausubel et al. teach this method should be conducted at an elevated temperature under acidic conditions to cleave an Asp-Pro bond between the component domains (page 16.4.13). Ausubel et al. teach that it is often advantageous to remove a carrier protein moiety from the protein of interest in order to do biochemical and functional analyses (page 16.4.2).

At the time of the invention, the ability to cleave an insoluble fusion protein by acid hydrolysis at an Asp-Pro bond was known in the art. For example, Better teaches acid cleavage of a human osteogenic protein subunit D (Bone D) polypeptide-bacterial/permeability-increasing protein (BPI) fusion at an Asp-Pro bond by acid hydrolysis using a variety of pHs and elevated temperatures (columns 19 and 20). The Bone D-BPI fusion protein was expressed in *E. coli*, resulting in the formation of inclusion bodies (column 18). Better teaches that, following acid hydrolysis and elevated temperature treatment of the fusion protein, the Bone D protein remained insoluble, while the BPI was solubilized, allowing isolation of the cleaved BPI from the acid supernatant (column 19, lines 52-58).

At the time of the invention it would have been obvious to one of ordinary skill in the art to combine the teachings of Smit et al., Nomellini et al., Ausubel et al., and Better for a method of cleaving a fusion protein comprising a *Caulobacter crescentus* S-layer protein fragment as taught by Smit et al. fused to BPI using acidic conditions and optionally elevated temperatures to cleave an Asp-Pro bond between the component domains, wherein the *Caulobacter crescentus* S-layer protein fragment as taught by Smit et al. remains insoluble and the BPI is solubilized. One would have been motivated for such a

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method in order to cleave the fusion protein and allowing isolation of the cleaved BPI from the *Caulobacter crescentus* S-layer protein fragment because of the teachings of Smit et al. and Better. One would have a reasonable expectation of success for a method of cleaving a fusion protein comprising a *Caulobacter crescentus* S-layer protein fragment as taught by Smit et al. fused to BPI using acidic conditions and optionally elevated temperatures to cleave an Asp-Pro bond between the component domains, wherein the *Caulobacter crescentus* S-layer protein fragment as taught by Smit et al. remains insoluble and the BPI is solubilized because of the teachings of Smit et al., Nomellini et al., Ausubel et al., and Better. Therefore, claims 1-6, drawn to a method for cleaving a fusion protein as described above, would have been obvious to one of ordinary skill in the art.

Applicant argues (beginning at the bottom of page 6 of Paper No. 19) the cited references fail to disclose the fragment of the *Caulobacter crescentus* S-layer protein that would remain insoluble after cleavage at low pH given the knowledge that the full length *Caulobacter crescentus* S-layer protein is soluble at low pH. Applicant cites (Nomellini et al. *J Bacteriol* 179 :6349-6354) in support of their assertion. Applicant argues that this unexpected property is evidence of nonobviousness. Applicant's argument is not found persuasive.

The amendment to claim 1 necessitated the inclusion of the teachings of Nomellini et al. As described above, Nomellini et al. teach that RsaA can be expressed as a precipitated protein that is resistant to acid solubilization. Thus, in view of the teachings of Nomellini et al., one of ordinary skill in the art would not expect the fusion protein of Smit et al. (comprising an insoluble fragment of a *Caulobacter crescentus* S-layer protein, e.g., a fusion-protein comprising amino acids 782-1026 of a *Caulobacter crescentus* S-layer protein, expressed using an S-layer shedding mutant) to be soluble at low pH, i.e., one of skill in the art would expect the fusion protein of Smit et al. to be insoluble at low pH. As such, the ability of the fusion protein of Smit et al. comprising an insoluble fragment of a *Caulobacter crescentus* S-layer protein, e.g., a fusion protein comprising amino acids 782-1026 of a *Caulobacter crescentus* S-layer protein, to remain insoluble even at low pH is not an unexpected property as asserted by applicant and it is the examiner's position that, in view of the teachings of Smit

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et al., Nomellini et al., Ausubel et al., and Better, claims 1-6 would have been obvious to one of ordinary skill in the art at the time of the invention.

**Conclusion**

**[10] Status of the claims:**


- Claims 1-6 are pending.
- Claims 1-6 are rejected.
- No claim is in condition for allowance.

**THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (703) 308-3934. The Examiner can normally be reached Monday-Friday from 7:00 am to 5:00 pm. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX number for submission of official papers to Group 1600 is (703) 308-4242. Draft or informal FAX communications should be directed to (703) 746-5078. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman  
Patent Examiner  
Art Unit-1652

  
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